QBD Approach To Analytical RP-HPLC Method Development And Its Validation For Estimation Of Quetiapine Fumarate In Bulk And Pharmaceutical Formulation

Ms. Priyanka Wadafale[1], Ms. Jyoti Kadam[2], Dr. Madhuri Shelar[3], Dr.Ganesh Andhale[4], Dr. Nalanda Borkar[5]

[1, 2 ,3]Dept of Quality Assurance Technique,

[4, 5]Dept of Pharmaceutical Chemistry

[1, 2, 3, 4, 5] Alard college of Pharmacy, Marunji, Near Rajiv Gandhi, IT Park, Hinjewadi, Pune, Maharashtra, 411028

Abstract- *The present study was aimed to develop and validate simple, sensitive, precise, economic and accurate reverse phase high performance liquid chromatography (RPHPLC) method for determination of Quetiapine Fumarate in Bulk and Pharmaceutical Formulation by QbD Approach the method was validate as per ICH guideline. Quetiapine is an antipsychotic medicine that is used to treat schizophrenia. Chromatographic separation was achieved on Grace Chromoline C8, (150 mm x 4.6 mm, 5 µm) at room temperature. The mobile phase consisted of Methanol: Acetonitrile: Ammonium formate buffer (60:10:30) at a flow rate 1 ml/min and UV-detection was monitored at 289nm. Injection volume of 40 ppm and total run time of 10.000 minutes. Retention time of Quetiapine Fumaratewas found to be 8.691 min, r2 value were 0.9994 and linearity range was 10ppm to 60ppm. The method was developed for accuracy, linearity, precision, recovery and stability in complies and stability in complies with ICH guideline.*

Keywords- Quality by Design, Quetiapine Fumarate, RPHPLC, UV, Design Expert Software

I. INTRODUCTION

Quetiapine Fumarate is chemically (2E)-but-2-enedioic acid; bis(2-[2-(4-{2-thia-9-azatricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,9,11,13-heptaen-10-yl}piperazin-1-yl)ethoxy]ethan-1-ol).Quetiapine is an antipsychotic medicine that is used to treat schizophrenia, major depression, and bipolar disorder.Quetiapine is a dibenzothiazepine derivative with antipsychotic property. Quetiapine fumarate antagonizes serotonin activity mediated by 5-HT 1A and 5-HT2 receptors. With a lower affinity, this agent also reversibly binds to dopamine D1 and D2 receptors in the mesolimbic and mesocortical areas of the brain leading to decreased psychotic effects, such as hallucinations and delusions. In addition, quetiapine also binds to other alpha-1, alpha-2 adrenergic and histamine H1 receptors.

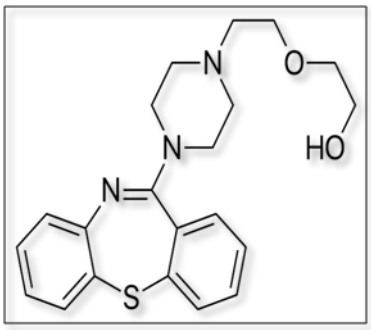

Figure 1: Structure of Quetiapine Fumarate

II. MATERIAL AND METHOD

Material:

A. Instruments:

HPLC (Shimadzu), Double beam UV-VIS spectrophotometer (UV-1800, Thermo, Japan) pH Meter (Chemiline), Balance (Labindia), Sonicator (Rolex).

Reagents and Materials:

Quetiapine Fumarate API, Formulation: Quetiapine Tablets 50 mg.

Chemicals- Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Ammonium formate buffer, Sodium Hydroxide, Distilled Water.

Methods:

B. Preparation of standard stock solution:

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with Diluent get concentration of solution 100 µg/ml. Further 4 ml of this solution was diluted to 10 ml with mobile phase to get concentration of solution 40 µg/ml.

C. Selection of detection wavelength:

From the standard stock solution (20 µg/ml) further dilutions was made using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the drug showed linear, stable and considerable absorbance at 289 nm.

D. Preparation of sample solution:

20 tablets was weighed and triturated to powder. A quantity of powder equivalent to 10 mg of Quetiapine Tablets was transferred to a 10 ml volumetric flask containing 10 ml of methanol. Furthermore resulting sample stock solution was filtered with syringe filter and the volume was made up with mobile phase the to get concentration of 1000 µg/ml. Further dilutionwere made to get concentration 20 µg/ml.

Trials given by Design Expert software

Standard concentration of Quetiapine was taken 40 µg/ml. Central Composite design gave 12 run at different pH, Solvent proportion two Solvent Combination with 12 runs for each Solvent Combination.

Table 1: Run Suggested by Software for each Solvent

Sr. No	Mobile Phase Composition (Organic Phase, v/v)	pH of Buffer mmol/L	Flow Rate (mL/min)
1	60.00	6.00	0.90
2	50.00	7.00	1.10
3	50.00	5.00	0.90
4	60.00	6.00	1.10
5	60.00	5.00	1.00
6	40.00	7.00	1.00
7	40.00	5.00	1.00
8	60.00	7.00	1.00
9	50.00	5.00	1.10
10	40.00	6.00	0.90
11	50.00	7.00	0.90
12	40.00	6.00	1.10

OPTIMIZATION RESULT:

Screening design for suitable chromatographic condition

Determination of chromatographic condition is based on peak parameters of drug.

After taking runs on HPLC, we got following results of different mobile phase with different pH and different flow rate. To have better understanding the peak properties used remarks like Extremely Satisfactory, Satisfactory, More Satisfactory, partially Satisfactory and Dissatisfactory.

Results of various trials, having organic phase composition 60 % v/v are shown in following tables.

Table 2: Runs performed at mobile phase (60:40 v/v) with aqueous phase pH 5.00.

Sr. no.	Composition	Observation	Remarks
1	Buffer: Methanol	Less peak asymmetry with more theoretical plates and good retention time	Partly Satisfactory
2	Water : Methanol	Good Peak Properties but Resolution is not Good	Partly Satisfactory
3	Water: Acetonitrile	The peak of lisinopril not appeared	Dissatisfactory

Table3: Runs performed at mobile phase (60:40 v/v) with aqueous phase pH 6.00.

Sr. no.	Composition	Observation	Remarks
1	Buffer: Methanol	Greater peak Asymmetry and lower theoretical plates	Partially satisfactory
2	Water : Methanol	Less peak asymmetry but less theoretical plates	Satisfied
3	Water: Acetonitrile	Resolution of Peaks is not good	Very Dissatisfactory

Table 4: Runs performed at mobile phase (60:40 v/v) with aqueous phase pH 7.00.

Sr. no.	Composition	Observation	Remarks
1	Buffer: Methanol	Good peak properties, less retention time with more theoretical plates and less asymmetric factor	Extremely Satisfactory
2	Water : Methanol	Lower theoretical plates and less peak height	Satisfactory
3	Water : Acetonitrile	Only one peak appeared (Amlodipine) another peak is very small (Lisinopril)	Dissatisfactory

Results of various trials, having organic phase composition 50 % v/v are shown in following tables.

Table 6: Runs performed at mobile phase (50:50 v/v) with aqueous phase pH 5.00.

Sr. no.	Composition	Observation	Remarks
1	Buffer: Methanol	More retention time	Not satisfactory
2	Water : Methanol	Greater peak asymmetry	Not satisfactory
3	Water : Acetonitrile	Very Small Peak appeared	Not satisfactory

Table 7: Runs performed at mobile phase (50:50 v/v) with aqueous phase pH 7.00.

Sr. no.	Composition	Observation	Remarks
1	Buffer: Methanol	Greater peak Asymmetry and lower theoretical plates	Partially satisfactory
2	Water : Methanol	Less peak asymmetry but more retention time	Satisfactory
3	Water: Acetonitrile	Peak not appeared	Very Dissatisfactory

Results of various trials, having organic phase composition 40 % v/v are shown in following tables.

Table 8: Runs performed at mobile phase (40:60 v/v) with aqueous phase pH 5.00.

Sr. no.	Composition	Observation	Remarks
1	Buffer: Methanol	More retention time	Not satisfactory
2	Water : Methanol	Broad Peak Appeared with average Theoretical Plates	Partly Satisfactory
3	Water: Acetonitrile	More retention time	Not satisfactory

Table 9: Runs performed at mobile phase (40:60 v/v) with aqueous phase pH 6.00.

Sr. no.	Composition	Observation	Remarks
1	Buffer: Methanol	Peak Properties are not Good	Not satisfactory
2	Water : Methanol	Peak Tailing observed	Not satisfactory
3	Water: Acetonitrile	More retention time	Not satisfactory

Table 10: Runs performed at mobile phase (40:60 v/v) with phase pH 7.00.

Sr. no.	Composition	Observation	Remarks
1	Buffer: Methanol	More Retention Time	Not satisfactory
2	Water : Methanol	More Retention Time	Not satisfactory
3	Water: Acetonitrile	More Retention Time	Not satisfactory

Table 11: Trials performed on C18 column at mobile phase (60:40 v/v) with aqueous phase pH 7 are extremely Satisfactory. Design expert has optimized the following chromatographic conditions with respect to desirability value.

Sr. No	Mobile Phase Composition (Organic Phase, v/v)	pH of Aqueous mmol/L	Flow Rate (mL/min)	Retention Time (Min)	Asymmetry	Theoretical Plates
1	60.00	6.00	0.90	8.878	1.423	9899
2	50.00	7.00	1.10	9.235	1.14	7214
3	50.00	5.00	0.90	11.634	1.524	7894
4	60.00	6.00	1.10	8.684	1.387	10214
5	60.00	5.00	1.00	8.699	1.644	9962
6	40.00	7.00	1.00	13.502	1.23	3246
7	40.00	5.00	1.00	11.761	1.687	4651
8	60.00	7.00	1.00	8.701	1.12	10256
9	50.00	5.00	1.10	9.365	1.628	7001
10	40.00	6.00	0.90	17.572	1.411	5621
11	50.00	7.00	0.90	11.741	1.19	7502
12	40.00	6.00	1.10	13.74	1.345	6521

This methodology is initially based on constructing a desirability function for each individual response. The scale of individual desirability function ranges between i= 0, for completely undesirable response and i =1, for fully desired response. Selection of trial was based on maximum desirability value. Therefore, first trial which was having desirability one (i=1) selected for method optimization.

Table 12: Optimized trials suggested by software based on desirability value

Sr. no.	Amount of Methanol	pH of Aqueous	Flow rate	Retention time	Tailing factor	Theoretical plates	Desirability
1	61.00	7.00	1.10	7.6729	1. 15	9877.8	0.964

Optimized chromatographic conditions

Mobile phase: Methanol: Water (61: 39 v/v), pH of buffer: 7.00, Analytical column: C_{18} column Waters XBridge (4.6× 250mm id. particle size 5µm), UV detection: 289 nm, Injection volume: 10 µL, Flow rate: 1.10 mL min^{-1}, Temperature: Ambient, Run time: 10 min.

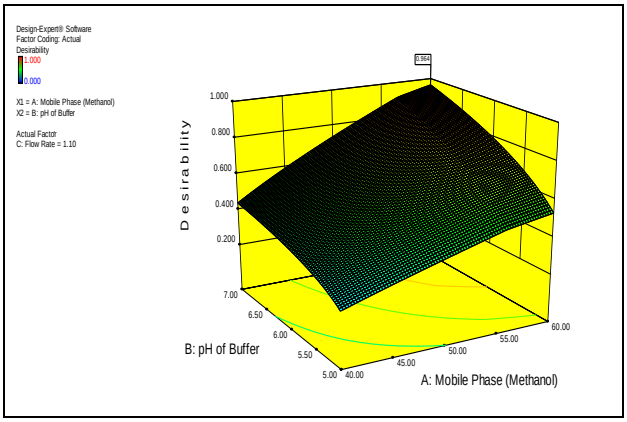

Figure 2: Desirability Value

Effect of independent variables on retention time (X):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 20.17, *p* value less than 0.005 and R^2 value of 0.8832. There is only a 0.04% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 8.96 and 0.8395 respectively. The model for response X (Retention time) is as follows:
The equation for response surface quadratic model is as follows

Retention Time = +31.62871 - 0.25516 * Mobile Phase (Methanol) + 0.14000 * pH of Buffer - 8.75125 * Flow Rate (1)

Fig.3 shows a graphical representation of pH of Aqueous Phase (B) and amount of Methanol (A), while flow rate (C) is maintained constant at its optimum of 1.10 mL min^{-1}. Change in pH of buffer showed slightly change in retention time (X), also increase in amount of Methanol showed decreases the retention time.

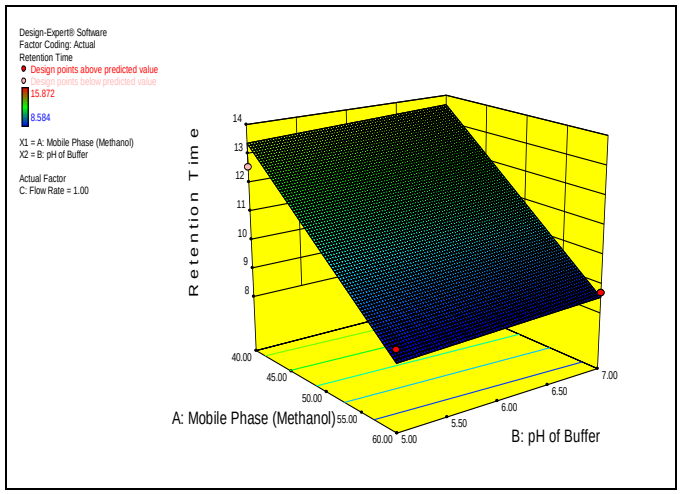

Figure 3:Three-dimensional plot for retention time as a function of pH of buffer and amount of Methanol. Constant factor (flow rate- 1.10 mL min^{-1})

Fit summary: Linear model was suggested by the software.

ANOVA: ANOVA of developed Full three level factorial model for retention time (Y_1).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant.

In this case A and B are significant model terms.

Table 13:Significance of *p* value on model terms of retention time

Model terms	*p* value	Effect of factor	Remarks
A	0.0001	52.09	Significant
B	0.16	0.6974	Insignificant
C	0.0358	6.13	Significant
Overall model	0.0004	-	Significant

Effect of independent variables on tailing factor (Y):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 45.65, *p* value less than 0.005 and R^2 value of 0.9448. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 3.91 and 0.9241 respectively. The model for response

Asymmetric Factor =+ 2.86821 - 1.23750E - 003 * Mobile Phase (Methanol) - 0.22538 * pH of Buffer - 0.060000 * Flow Rate

Fig.4. shows a graphical representation of pH of buffer (B) and amount of Methanol (A), while flow rate (C) is maintained constant at its optimum of 1.10 mL min^{-1}. A decrease in pH of aqueous phase decrease the tailing factor, it is synergistic effect on response (Y) while increase in amount of Acetonitrile showed no drastic change in the asymmetry.

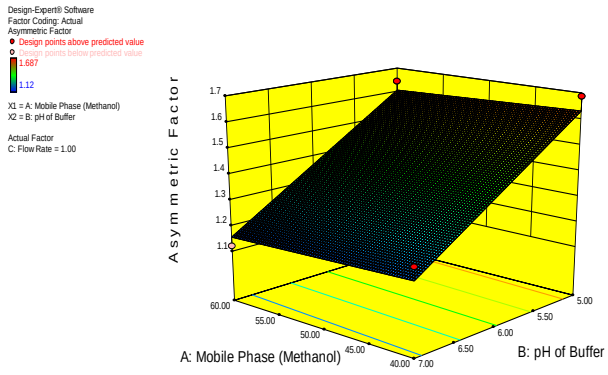

Figure 4:Three-dimentional plot for tailing factor as a function of pH of buffer and amount of methanol. Constant factor (flow rate- 1.10 mL min^{-1})

Fit summary: Response Surface Linear Model was suggested by the software.

ANOVA: ANOVA of developed CCD model for tailing factor (Y_2).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant.

In this case B is significant model terms.

Table 14:Significance of *p* value on model terms of tailing factor

Model terms	*p* value	Effect of factor	Remarks
A	0.5392	1.225E-003	Insignificant
B	0.0001	0.41	Significant
C	0.7638	2.880E-004	Insignificant
Overall model	0.0001		Significant

Effect of independent variables on theoretical plates (Z):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 21.92, *p* value less than 0.005 and R^2 value of 0.8915. There is only a 0.03% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 11.82 and 0.8509 respectively. The model for response Z (theoretical plates) is as follows:

Theoretical Plates = -4259.08333 + 253.65000 * Mobile Phase (Methanol) -61.25000 * pH of Buffer + 42.50000 * Flow Rate

Fig.5. shows a graphical representation of amount of Methanol (A) and pH of buffer (B), while flow rate (C) is maintained constant at its optimum value 1.10 mL min^{-1}. A decrease in pH of buffer showed not a significant effect on number of theoretical plates (Z), while increase in amount of Acetonitrile showed increases response.

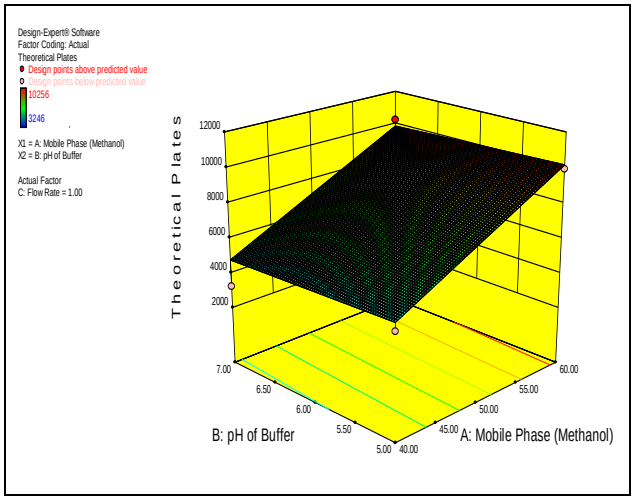

Figure 5:Three-dimentional plot for theoretical plates as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1 mL min^{-1})

Fit summary: Linear model was suggested by the software

ANOVA : ANOVA of developed CCD model for theoretical plates (Y_3).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case A value is significant model terms.

Table 15:Significance of *p* value on model terms of theoretical plates

Model terms	*p* value	Effect of factor	Remarks
A	0.0001	5.147E+007	Significant
B	0.6208	2.080E+005	Insignificant
C	0.9895	144.50	Insignificant
Overall model	0.0003		Significant

III. RESULTS AND DISCUSSION

1. Optimized chromatographic conditions

The HPLC Validation of Optimized result of Quetiapine is at 7.0 pH, Mobile Phase of MeOH: Acetonitrile: Ammonium formate buffer (60:10:30 v/v/v) at Maximum Wavelength 289nm

2. Validation

1. *Linearity:*

The method gave a linear response to Quetiapine drug within the concentration range of 10 – 60 µg/mL with r2 = 0.9994 as shown in figure 6. The chromatograms were obtained and peak area was determined for each concentration of drug solution and given in Table 16. Calibration curve of Quetiapine Fumarate was constructed by plotting peak area vs applied concentration of and regression equation was computed. The slope, intercept, and correlation coefficient were also determined and are shown in Table 17. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range which are presented in Fig No.7.

Table 16: Linearity Result of Quetiapine Fumarate

Sr.No.	Concentration (µg/ml)	Peak Area
1	10	42515
2	20	90413
3	30	130854
4	40	182023
5	50	223564
6	60	270688

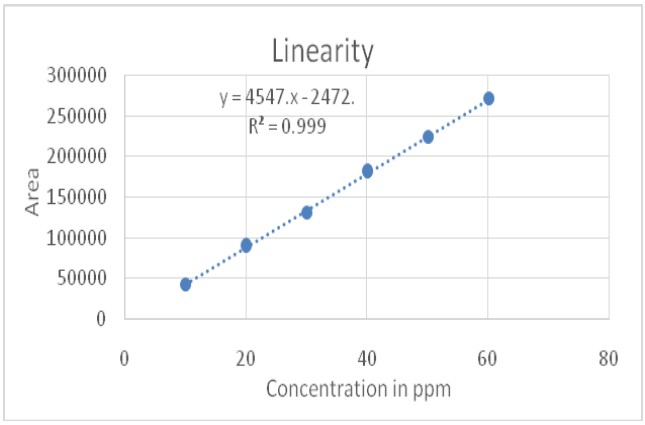

Figure 6: Calibration Curve of Quetiapine Fumarate

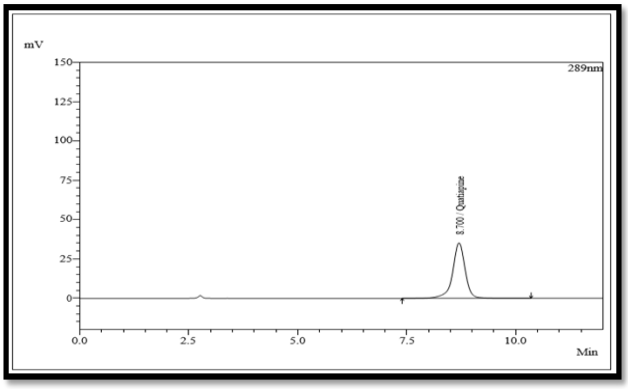

Figure 7:A typical chromatogram of injection 1

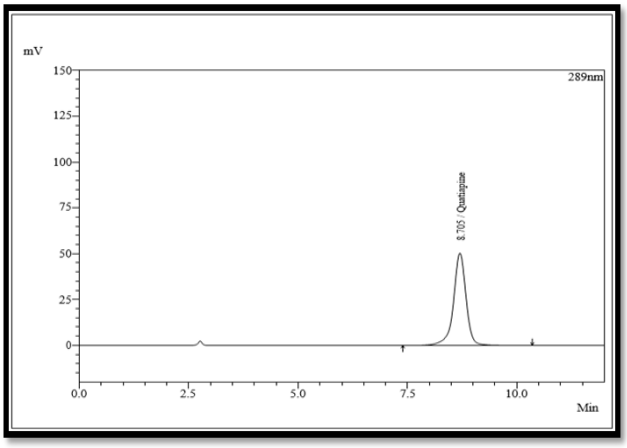

Figure 8: A typical chromatogram of injection 2

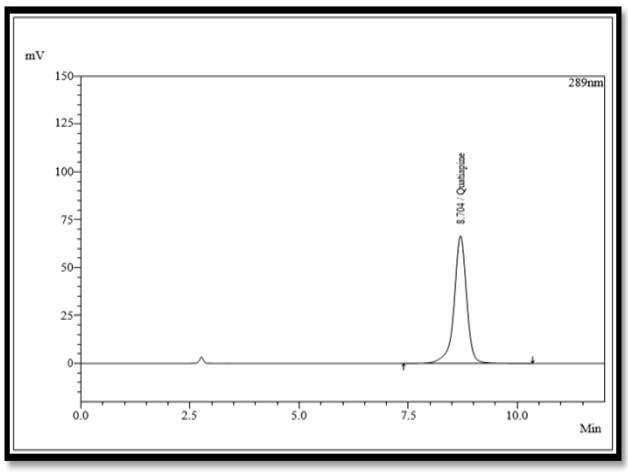

Figure 9: A typical chromatogram of injection 3

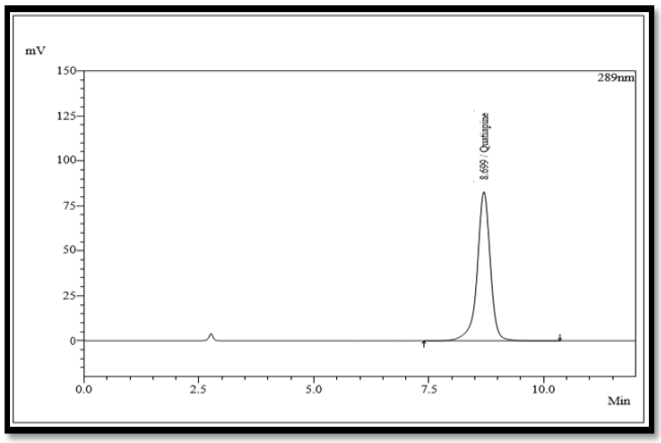

Figure 10:A typical chromatogram of injection 4

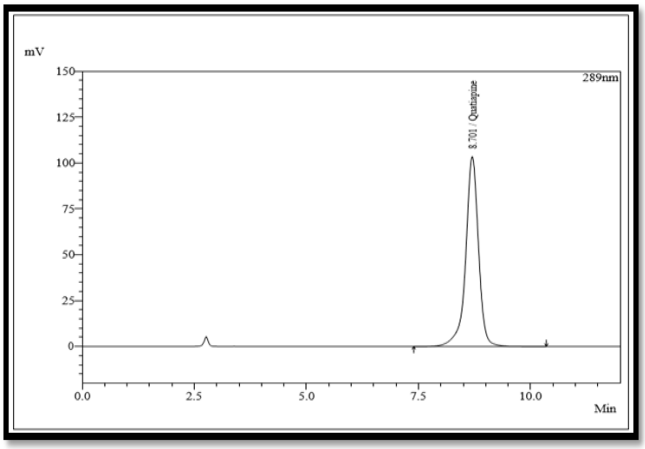

Figure 11: A typical chromatogram of injection 5

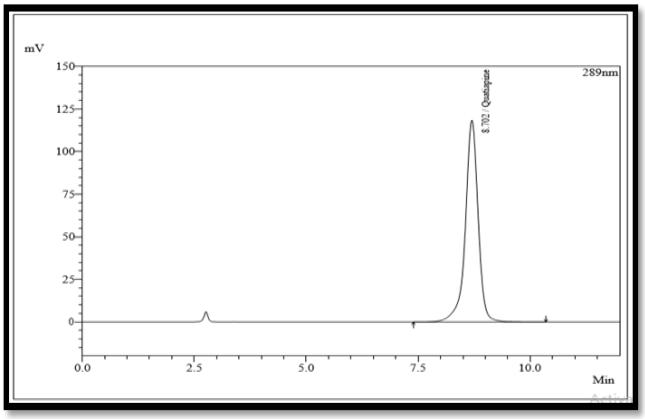

Figure 12: A typical chromatogram of injection 6

Table 17: Characteristic Parameters

Sr.no.	Parameter	Results
1	Calibration range (µg/ml)	10 – 60
2	Regression equation (y*)	y = 4547.1x –2472.5
3	Slope (b)	2472.5
4	Intercept (a)	4547.1
5	Correlation coefficient(r2)	0.9994

2. System Suitability:

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 40 µg/ml. The results which are given in Table No 18.Were within acceptable limits.

Table 18: System Suitability Studies of Quetiapine By HPLC Method.

Sr.no.	Parameter	Value
1	Retention time	5.910
2	Area	183341
3	Asymmetry (NMT 2.0)	1.12
4	Theoretical plates (NLT 2000)	13445
5	% RSD of Peak Area (NMT 2.0)	1.17

3. Specificity:

Chromatogram of blank was taken as shown in Fig No. 13. Chromatogram of Quetiapine showed peak at a retention time of 5.910 min. The mobile phase designed for the method resolved the drug very efficiently. The Retention time of Quetiapine sample (Tablet) was 5.986 min. The wavelength 289nm was selected for detection because; it resulted in better detection sensitivity for the drug. The peak for Quetiapine Fumarate from the injection was Quetiapine Fumarate.

Table 19: Specificity Of Quetiapine Fumarate By HPLC Method.

Concentration	API Area	Tablet Area
40	182923	179989
40	185124	185441
40	184720	183945
40	185467	185342
40	184200	182103
40	189355	184113
Mean	185298	183489
SD	2177.08	2098.20
RSD	1.17	1.14

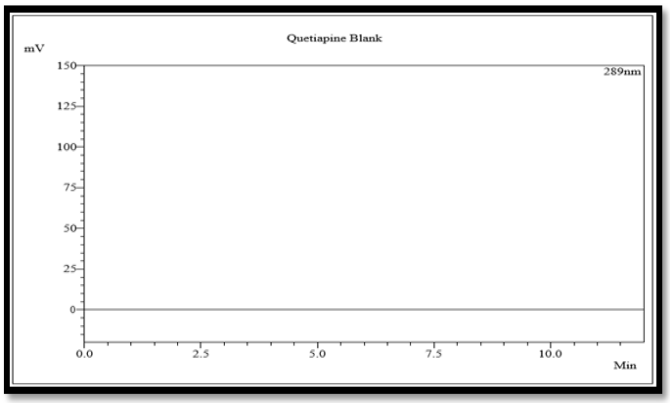

Figure 13: A typical chromatogram of Blank

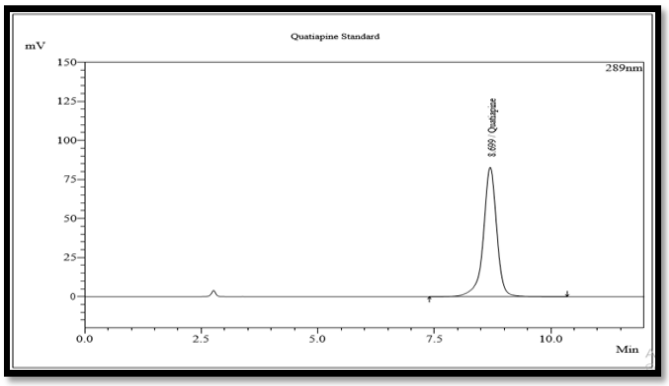

Figure 14: A typical chromatogram of Quetiapine Standard [Concentration 40ug/ml]

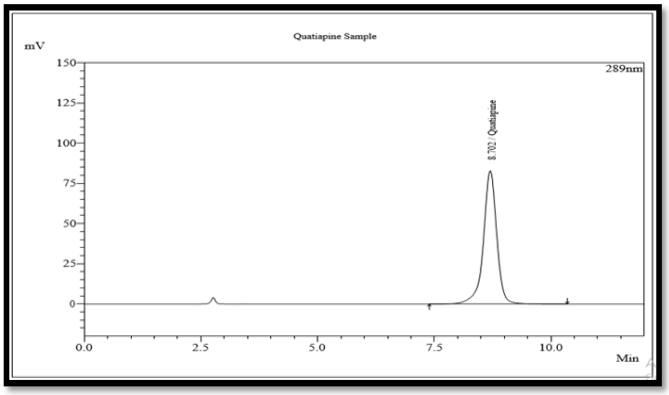

Figure 15: A typical chromatogram of Quetiapine Sample [Concentration 40ug/ml]

5. Accuracy:

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analysed samples of Quetiapine (40 µg/ml) were spiked with 50, 100, and 150 % extra Quetiapine standard and the mixtures were analysed by the proposed method. Standard deviation of the % recovery and % RSD were calculated and reported in Table No 20.

Table 20: Accuracy of Quetiapine Fumarate

Sr. No.	Concentration	Peak Area	recovery%	Mean	SD	RSD
1	40	182299	98.46	183123	736.1143	0.401977
2	40	183356	101.02			
3	40	183715	101.17			
4	50	226085	99.86	226049	201.877	0.089307
5	50	226231	100.89			
6	50	225832	98.42			
7	60	273412	100.65	273249	208.5402	0.076319
8	60	273321	100.12			
9	60	273014	99.98			

6. Robustness:

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of wavelength and mobile phase flow rate by 1.0 ml/min (0.9 and 1.1 ml/min) had no significant effect on the retention time and chromatographic response of the 20 µg/ml solution, indicating that the method was robust. The results are shown in Table No.21 & 22.

Table 21: Robustness of Quetiapine Fumarate

Conc. (µg/ml)	Area	
	289 nm	292 nm
40	182923	139905
40	185124	138579
40	184720	136584
40	185467	138668
40	184200	136277
40	189355	135589
Mean	185298	137600
SD	2177.08	1687.53
RSD	1.17	1.23

Table 22: Robustness of Quetiapine Fumarate

Conc. (µg/ml)	Flow rate	
	0.8 ml/min	1.0 ml/min
40	129653	182923
40	126583	185124
40	126720	184720
40	126467	185467
40	126300	184200
40	129355	189355
Mean	127513	185298
SD	1551.24	2177.08
RSD	1.22	1.17

REFERENCES

[1] Beckett A.H., Stenlake J.B, Practical Pharmaceutical Chemistry. 4th ed. Part 2. Publication of New Delhi, (2004) pp.275-300.

[2] Kar A. Pharmaceutical Drug Analysis. 2nd ed. New age international limited publishers, pp. 452-474, 2005.

[3] Juran, J. M. Juran on quality by design: the new steps for planning quality into goods and services. The Free Press: USA, pp. 287-300, 1992.

[4] Skoog D., Holler, J., Crouch, S., Principles of Instrumental Analysis. 6th ed. Belmont, CA: Thomson Brooks/Cole, pp. 169–173, 2007.

[5] L.D. Torbeck, ―OOS, OOT, OOC, and OOSC,‖ Pharmaceutical Technology, vol.35, no.10, pp.46–47,2011.

[6] Chatwal, G.R., Anand S.K., Instrumental Methods of Chemical Analysis. 5th ed. Himalaya Publishing House; Delhi, pp.2.150, 2.566-2.585, 2.624-2.630,2007.

[7] ICH – Q2B. (1996). Analytical Guidelines on Conference on Analytical Method Validation. International Conference on Harmonization (ICH), pp. 1518.

[8] British Pharmacopoeia. (2007). Apendices-III. Chromatographic Separation Techniques. The Stationary Office behalf of the Medicines and HealthcareProducts Regulatory Agencies: London, 5. pp.189-190.

[9] The Merck Index, Merck & Co. Inc. 14th ed. White house station. NJ. pp. 4781, 491, 4935, 6528, 2006.

[10] USP 2007 NF 25Volume II, DI-Advice for the Patient: Drug Information in Lay Language Guidance.

[11] Ashish Baldi, Ashutosh Kumar, PatidarandJyotsana, Sanadya. Method Development and Validation for Estimation of Quetiapine Fumarate by RP-HPLC. Asian J. Research Chem. 2010; 3(3): 604.

[12] Bently and Drivers. Text book of pharmaceutical chemistry,8th Edition, oxford university press,London , 1985: 1-3.33

[13] Sravan Kumar G. et al. / International Journal of Biological & Pharmaceutical Research. 2011; 2(1): 27-33.

[14] Chatwal GR and Anand SK. Instrumental Methods of Chemical Analysis, Himalaya Publishing House, New Delhi, 2004: 21-57.
[15] David Harvey. Modern analytical chemistry, Mcgraw hill publishers, Toronto, 2000: 38-47.
[16] Guidance for Industry: Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls
[17] Documentation; Draft Guidance. Rockville, MD. US FDA. 2000.
[18] International Conference on Harmonization, "Q2A: Text on Validation of Analytical Procedures," Federal Register, 1995;60(40): 11260-11262.
[19] International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," Federal Register 1997; 62(96): 27463–27467.
[20] Lloyd R Snyder, Joseph J, Kirkland Joseph, Glash, Chatwal Anand. Mass spectrometry Practical HPLC method development.Instrumental analysis. 2007: 2.624-2.639.
[21] Remington. The science and practice of pharmacy, volume I, 21st edition, Lipincott Williams & Wilkins publishers, Newyork, 2007: 621-626.
[22] Sethi PD. HPLC-Quantitative analysis of pharmaceutical formulations, CBS publishers& distributors, New Delhi, 2006: 118-120.
[23] Shabir GA, Lough WJ, Shafique AA, Bradshaw TK. Evaluation and application of best practice in analytical method validation. J LiqChromatogrRelat Technol. 2007; 30: 311-33.
[24] Shabir GA. Validation of HPLC methods for Pharmaceutical Analysis: Understanding the differences and similarities between Validation reqirements of the U.S Food and Drug administraton, The U.S Pharmacopoiea and International Conference on Hanmonization. J Cromatogr A. 2003; 987: 57-66.
[25] Skoog, Holler, Nieman. Principles of instrumental analysis, 5th edition, Harcourt college publishers, fortworth, 2004: 725-739.
[26] Synder KL, Krikland JJ and Glajch JL. Practical HPLC Method Development, 2nd Edition, Wiley-IntersciencePublication,USA, 1983: 1-10.
[27] Yuri Kazakevin, Rosario Lobrutto. HPLC for pharmaceutical analysis, Wiley interscience, John wiley& sons Inc. New jersey, 2007: 347-490.